The Use of Water and Ice with Bactericide to Prevent Onboard and Onshore Spoilage of Refrigerated Megrim *(Lepidorhombus whiffiagonis)*

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ABSTRACT

This study investigates the effectiveness of ozonated water and flake ice (combined Petfrost system) to increase the quality and stability of fresh megrim on fishing boats. The captured fish were washed, placed in plastic boxes, covered with flake ice and refrigerated at 2ºC for up to two-weeks onboard and, thereafter, for 11 days onshore. The experiments employed sterile, filtered and ozonated water at a concentration of 2 ppm for washing the fish and making the flake ice. The results are compared with samples from a traditional treatment consisting of water and flake ice of marine origin. Fish were caught in four different hauls, which took 14, 12, 8 and 3 days in being landed. Subsequently, fish were stored for 1, 5, 7 and 11 days at 3ºC. The different treatments were evaluated using sensory, microbiological and chemical techniques. Fish treated with ozone always showed the best quality. Megrim treated with ozone was still suitable for consumption after 14 days on board, and megrim stored for 12, 8 and 3 days on board could be stored for five further days in the ice state once landed with an acceptable quality. In contrast, control fish were not suitable for consumption if stored for longer than three days on board.The results indicate that treatment with water and ice flakes made from sterile and ozonated water maintains the quality of fresh megrim onboard fishing boats and upon arrival onshore.

Keywords. Megrim, ozonised water, flake ice, refrigeration, on board.

1. Introduction

The Codex Alimentarius defines ozone as an antimicrobial agent and disinfectant for use in foodstuffs, both in the water destined for direct consumption and in ice or substances for indirect consumption, such as the water used to preserve fish, agricultural products and other perishable foods (Pérez, Palacios, & Amigo, 2006). In 2001, the US department for Food and Drug Administration (FDA) listed ozone as a safe (GRAS: Generally Recognized as Safe) antimicrobial agent for use in direct contact with food products, including fish, meat and poultry (Mielcke & Ried, 2006). The Electric Power Research Institute and the Agriculture and Food Technology Alliance submitted a petition to the FDA demanding the use ozone in food processing without limitations (Graham, 2000). In response, the FDA amended the food additive regulations to include gaseous and aqueous ozone as an antimicrobial agent (Molloy, Hassenberg, Plöchl, Idler, Geyer, & Barnwes, 2001). This approval should encourage an increased use of ozone in food processing (Kim, Yousef, & Khadre, 2003).

Ozone at low concentration (0.01 ppm) is toxic for gram-positive and gram-negative bacteria (Mielcke et., 2006) and has an oxidation potential 1.5 times greater than chlorine (Lin & Yeh, 1993). The bactericide effect of ozone depends on the ozone concentration, the contact time, temperature and the production system used. Low ozone concentrations in solution decrease the half life of ozone in some cases and the bactericide effect is unobserved. However, when the concentrations of ozone in solution are sufficiently high (~2 ppm) the effect of ozonated water and ice can improve the quality of refrigerated fish (Pastoriza, Bernárdez, Sampedro, Cabo, & Herrera, 2007). The decomposition of ozone in air occurs at much lower rates than in solution (Glaze, 1986) and can react with other components on the surface of fish to produce a bactericide effect. Additionally, Ravesi, Licciardello, & Racicot (1987) reported that ozone may react with some components in sea water to produce a bactericidal ion or compound. The use of a gaseous ozone system provides fisherman with a practical alternative to improve catch quality and marketability (da Silva, Gibbs, & Kirby, 1998). The absence of adverse sensory effects and harmful oxidation by-products makes ozone a desirable antimicrobial agent in processing fish products for human consumption (Kim et al., 2003).

In Spain, the majority of fish catches sold at fresh fish markets comes from the Grand Sole fishing bank (North Atlantic). The trawlers typically spend two weeks at sea, during which the daily catch is stored in ice and refrigerated onboard. The storage time thus varies between 3 and 15 days, depending on which day the fish is caught, before arrival onshore and sale at auction. Among the marine flat fish, megrim is highly prized both by consumers and restaurants. The present study focuses on increasing the lifetime of megrim when refrigerated onboard. Two treatments are compared (i) washing of the fish with ozonated water followed by refrigeration inozonated flake ice, and (ii) the traditional treatment employing sea water for the washing and ice making. The quality and grade of freshness of the megrim once on land and stored for 11 days at 2-3ºC is also investigated.

2. Material and methods

2.1. Water and flake ice preparation

The Petfrost system was used to filter, sterilize and ozonate the water required for fish washing and flake ice production (Taboada, 2004). For both treatments, the flake ice was prepared onboard with a saline solution (0.10-0.15%):

(1)Control flake ice was prepared onboard in an ice machine using the homogenized saline solution (Icematic, Castel MAC, Castelfranco Veneto, Italia). This is termed control flake ice in the text. (2) Flake ice with bactericide was prepared from the saline solution using the Petfrost system with an ozone concentration of 2 ppm. This is termed Petfrost flake ice in the text.

2.2. Sample preparation

A total of 50 kg of megrim (length 22 ± 2 cm) caught by trawlers working in the North Atlantic during June 2006 was used for the experiments (Table 1). After the fish were landed, they were separated from the by-catch and washed with water from the Petfrost system for 3 seconds. Subsequently, the fish were placed in boxes with Petfrost flake ice in a 2:1 fish: ice ratio and stored onboard at 2ºC. The fish used in this work had been stored onboard and at sea for 14, 12, 8 and 3 days, and are identified in the text as batch 1, 2, 3 and 4, respectively. The sealed and refrigerated fish boxes were sent to the Marine Research Institute (IIM, Vigo, Spain) where additional ice was added to the control and Petfrost boxes in the same 2:1 fish: ice ratio, and then refrigerated at 2ºC. These samples were compared with control samples which used saline water only (0.10-0.15%) for washing and flake ice production. Laboratory analyses of the fish were carried out after 1, 5, 7 and 11 days after their arrival at IIM. For both the control and Petfrost samples, only the fish stored onboard for 8 and 3 days were stored for a further 11 days onshore since the catches which had spent 12 and 14 days onboard were rejected on the basis of chemical, microbiological and sensory analyses.

2.3. Proximate analysis

Moisture, crude protein, ash and crude fat were measured in triplicate following the procedures proposed by AOAC (1995). Moisture was determined by oven drying at 105ºC. Crude protein was determined by mineralization of the sample with sulfuric acid and a Se/Cu catalyst, followed by distillation, and then analyzed with 0.1N HCl using a 2300 Kjeltec Analyzer Unit (FossTecator, Höganäs, Sweden). Crude fat was measured by Soxhlet extraction of the fat with ethyl ether, and ash was determined by oven heating at 550°C for 24h.

2.4. Microbiological analysis

Under sterile conditions inside a vertical laminar-flow cabinet (Telstar, AV-100, Tarrasa, Spain), 10 g of megrim muscle was placed in a sterilized plastic filter bag (Seward, Thetford, Norfolk, UK) with 90 ml of peptone water. Appropriate dilutions were prepared following 1 min sample mixing in a stomacher blender (ITUL Instruments, 2997/400, Barcelona, Spain). The total number of aerobic microorganisms (total viable counts, TVC) was used as an indicator of the limit of product acceptance. The TVC analysis was performed on Plate Count Agar (Panreac, Barcelona, Spain) supplemented with 1% NaCl (Panreac) incubated at 17ºC for 3 days. Microbiological counts were expressed as log colony forming units per gram of sample ($CFUg^{-1}$).

2.4. Chemical determinations

Triplicate samples of homogenized fish muscle with distilled water (ratio 1:2) were analyzed using an electrode (Crison, Barcelona, Spain) to determine the pH variation. Total volatile nitrogenous bases (TVB-N) were determined according to Lücke and Geidel (1935) and Antonacopoulos (1960). The analysis was based on titration with 0.1N HCL using a soliution of a boric acid indicator (pH, 5.5) of a distillate of fish muscle triturate (10 g) in water and OMg (2 g) (Panreac, Barcelona, Spain). The results are expressed in mg TVB-N per 100g of muscle. Trimethylamine-nitrogen (TMA-N) values were determined by the picrate method (Dyer, 1945) employing a 7.5% trichloroacetic acid (Panreac) extract of fish muscle (20 g/100 ml). The concentration is expressed in mg TMA-N per 100g of muscle. Dissolved ozone concentration was measured with the indigo method of Bader, and Hoigné (1981), in which the ozone reacts selectively with the double carbon bond of the sulfonated indigo molecule. Therefore, the ozone measurement was not affected by the presence of hydrogen peroxide, organic peroxides, manganese ions or oxidized species in the aqueous medium (Kim et al., 2003).

2.5. Sensory analysis

For raw megrim analysis, 8 panelists were familiarized with the sensory analysis. The EU grading scheme (Council Regulation, 1996) was used to describe each attribute, choosing the attributes which avoided cutting or dissection. Gill color was not intense and differences were observed between individuals rather than between the treatment methods, and this attribute was thus excluded from the evaluation. Each single descriptor was associated with demerit points on a

scale of 0 to 3. A total of 19 demerit points were possible, and a Quality Index (QI) was calculated as the QI ratio = ss/19, where "ss" is the sum of the scores of each characteristic assessed. Thus QI ranged from 0 (best) to 1 (worst).

The sensory analysis of cooked fish was performed by 10 trained panelists and was based on color, smell, taste, texture and palatability after 3-5 min microwave cooking on a scale from 0 to 5 (Lupin, Glannini, Soulé, Davidovich, & Boeri, 1980). The fish was cooked whole, filleted and presented to the tasting panel. The total score for each of characteristic indicates the loss in quality of the product, with a possible maximum of 25. The Quality Index of Cooked fish (QIC) was calculated as the QIC ratio=cs/25, where "cs" is the sum of scores of each attribute of the cooked fish assessed. Thus, QIC ranged from 0 (best) to 1 (worst).

2.6. Statistical analysis of results

The results obtained for the control and Petfrost treatments with regard to storage time were subjected to an analysis of variance (Statistica 6.0 software) for each sampling period, using a Student's t-test with a significance level of 95%. Statistical differences shown in the tables are identifiable with different letters. Parameter correlations were also calculated using the statistical software.

3. Results and discussion

The approximate composition of megrim in g kg^{-1} ± SD was: water 782.6 ± 7.7; crude protein 190.6 ± 6.5 (total nitrogen x 6.25), crude fat 19.3 ± 0.5 and ash 14.2 ± 0.7.

3.1. Microbiological results

When compared to the untreated samples, the Petfrost treatment significantly reduced the bacterial population in the fresh megrim, with significantly lower ($p<0.05$) TVC of undesirable bacteria commonly present in refrigerated fish (Fig. 1). Upon arrival onshore, after having been refrigerated onboard for 14, 12, 8 and 3 days, the bacterial counts were 5.75, 5.01, 4.77 and 4.27 log CFU g^{-1}, respectively, for the Petfrost samples and 6.26, 6.08, 5.49 and 5.04 log CFU g^{-1} for the control samples. In all cases, the differences between treatments were significant. At the IIM (day 1) the fish were stored at 2ºC, and the control samples which had been onboard for 14 and 12 days were unsuitable for consumption since they surpassed the limit of bacterial content

established by European legislation (>1 million bacteria per gram). However, the Petfrost samples had values below this limit and significant differences were observed between the treated and control samples from the 12 and 14 day batches. After 5 days refrigeration, the control and Petfrost samples surpassed 1 million bacteria per gram with significantly different bacterial counts.

When the samples which had been stored onboard for 8 days arrived onshore (day 1 at IIM, in total 9 days storage), the total count of aerobes between the control and Petfrost treatments was significantly different, yet in both cases lower than 6 log units (5.49 and 4.77 log CFU g^{-1}, respectively). After 5 days storage at IIM at 2ºC, the samples again had significantly different values and the control samples were discarded (6.55 log units) whereas the treated samples were still within permitted limits (5.63 log units). Therefore, this Petfrost-treated fish was considered suitable for consumption after a total of 13 days refrigeration.

The control and Petfrost treated fish which arrived onshore after only 3 days onboard could be maintained refrigerated onshore for a further 5 and 7 days, respectively (a total of 8 and 10 days storage). However, subsequent analysis showed that the Petfrost samples could be refrigerated onshore for 11 days (14 days in total), at which point the total aerobic count was 5.9 log units. The corresponding control surpassed permitted limits (6.9 log units). In addition, bacterial counts were significantly different for the control and Petfrost samples after 1, 5, 7 and 11 days refrigerated storage at IIM. In summary, megrim treated onboard with ozonated water and flake ice (Petfrost system) can be maintained refrigerated on land for a further 1, 5, 7 or 11 days, depending on the day of catch.

The antimicrobial affect of ozone was also noted for gutted mackerel by Haraguchi, Simidu, & Aiso (1969), who observed a logarithmic reduction in viable bacteria after submerging the fish in an aqueous solution of 3 % NaCl containing 0.6 ppm ozone for 30-60 minutes. Crapo, Himelbloom, Vitt, & Pedersen, (2004) observed that ozonated water (0.6-1.5 ppm) was an effective bactericide for steel and plastic surfaces in fresh fish preparation rooms. Furthermore, da Silva et al., 1998) noted a 1 log cfu cm^{-2} reduction in specific microorganisms in skin and muscle when fish was treated daily with ozone. Other authors concluded that ozone reduced contamination on the surface of fresh vegetables, and suggested that washing with ozonated water could be an effective disinfectant for foods (Selma, Beltran, Chacon-Vera & Gil, 2006; Rico, Martin-Diana, Frias, Henehan, & Barry-Ryan, 2006; Zambuchini, Giosia, & Sturba, 2006; Karaca & Velioglu, 2007). Restaino et al., 1995 reported that ozonated water was highly effective in killing both gram-negative and gram-positive food-associated bacteria. The microorganisms usually responsible for food spoilage are gram-negative bacteria, which cause tissue breakdown

(Barriga, Trachy, Willomot, & Simard, 1991). In this work, it can be shown that ozonated water and ice was effective in reducing bacterial numbers on fresh fish, thus decreasing microbial spoilage and assuring food safety.

3.2. TMA-N

For the batches corresponding to 14, 12, 8 and 3 days after capture, the Petfrost fish showed significantly lower levels of TMA nitrogen (7.38, 1.91, 0.83 and 0.54 mg TMA-N/100g) than the control fish (12.0, 3.85, 2.76 and 0.61 mg TMA-N/100g), respectively (Fig. 2). Considering that the concentration limit established by European legislation is 12 mg/100g TMA-N (Directive 91/493/EEC), the 14 day control sample was unsuitable for consumption upon arrival onshore (12.00 mg) in contrast to the Petfrost fish (7.38 mg).

The TMA-N values measured on the first day onshore after 12 and 8 days onboard storage (13 and 9 days total storage) were within legal limits for the control and treated samples. However, significant differences were observed between both treatments. The increase in TMA-N levels after 5 days storage at the IIM rendered the 13 and 9 day control batches unsuitable (16.5 and 12.89 mg, respectively), yet suitable (8.27 and 1.33 mg) for the Petfrost samples. After 7 and 11 days refrigeration, only the Petfrost batch stored onboard for 8 days (a total of 15 and 19 days storage) had lower TMA-N values than the permitted limit (3.46 and 10.69 mg, respectively).

The fish from the batch refrigerated onboard for 3 days could be refrigerated for a further 11 days onshore (a total of 14 days storage) for the control and treated samples (5.24 and 2.95mg TMA-N/100g, respectively), whilst not exceeding permitted TMA-N limits. In this same batch, the amine values were significantly different for the control and Petfrost samples after 1, 5, 7 and 11 days onshore refrigeration at the IIM.

3.3. TVB-N

Similar trends as above were observed for TVB nitrogen (Table 2). When the fish arrived onshore (day 1) after 14 days onboard refrigerated storage, the Petfrost samples ahowed significantly lower levels of TVB (22.38, 13.40, 13.38, 7.96 mg TVB-N/100g) than the samples treated with sea water and ice (29.64, 18.30, 18.06, 8.30 mg TVB-N/100g). The presence of ozone in both water and flake ice thus had a favorable effect on TVB nitrogen for all fish regardless of the day of capture.

The legal limit of TVB-N is 30 mg/100g (Directive 95/149/EEC). Fish refrigerated for 7 days onshore and which had been previously stored on the trawler for 12 and 8 days (a total of 19 and

15 days storage) showed permissible TVB levels for the Petfrost sample (26.25 mg and 19.86 mg) but not for the control (41.69 mg and 36.0 mg).

Finally, the TVB levels in fish stored onboard for 3 days and analyzed on the day 1 onshore were within legal limits for both the control and treated batches. In all the control and treated samples, no significant differences in TVB levels were observed following refrigeration for 1, 5, 7 and 11 days and were within legal limits.

Variability in the nitrogen compounds in fish occurs as a result of both endogenous enzymatic and bacterial activity (Matches, 1982). TMA-N and TVB-N are not always adequate for quantifying the freshness of all fish species (Price, Melvin, & Bell, 1991; Civera, Turi, Bisio, Parisi, & Fazio, 1993). With regard to megrim, however, there is a good relationship between these two parameters and the level of freshness (Aubourg, Losada, Gallardo, & Barros-Velazquez, 2006) established according to European regulations (Civera, Turi, Parisi, & Fazio, 1995). In this study, and in agreement with Civera et al. (1995), both parameters indicate that the Petfrost system (ozonated water and flake ice) is more favorable than the control (saline water and flake ice) for megrim preservation when refrigerated for two weeks onboard followed by a further week on land or 11 days in some trials.

3.4. pH

The pH values varied according to the day the fish were caught, whereby samples with lower storage time, either onboard or on land, had a lower pH. The Petfrost-treated samples always had lower pH than the control samples (Table 3). On arrival onshore (day 1), no significant differences were observed in pH between the control and Petfrost samples for any of the batches, and all showed pH <7. After 5 days refrigerated on land, the samples from batches corresponding to 12 and 8 days onboard showed significant differences in pH for the Petfrost (7.00 and 6.79) and control samples (7.22 and 7.06), respectively. The pH was always significantly higher in the control samples. For the batch stored onboard for 3 days, no significant differences were observed in pH for the control and Petfrost samples after 1, 5, 7 and 11 days refrigeration on land, and in all cases the pH values were <7. Following fish capture, the pH of the muscle tissue for the majority of fish species is usually below 7. This is due to the presence of lactic acid from hydrolysis of the glycogen produced after death. Thereafter, bacterial action produces undesirable alkaline compounds, such as ammonia and TMA, which increase the pH to 7.0 and above, at which point the fish are usually discarded (Connell, 1980; Hebard, Flick, & Martin, 1982). In the present study, all samples with pH ~7 or less were deemed acceptable for consumption on the basis of the sensory characteristics of the fresh fish.

3.5. Sensory Results

The sensorial examination of the fresh fish followed the attributes described in Table 4 the samples with lowest values had a higher level of freshness, and corresponded to the batches which were stored onboard for the least number of days. In general, the Petfrost samples showed a higher sensorial quality throughout storage and had lower values than the control (Fig. 3). The microbiological data were used to establish the limit of acceptability of the fish. Applying the Pareto rule, only 20 % of the cases had a log value >6 in the interval 0-0.65, and no sample with a value of 0.65 or higher had log value below log 6. This threshold coincides with the limit of freshness in Mediterranean hake applying the ROC curve (Baixas-Nogueras, Bover-Cid, Veciana-Nogués, Nunes & Vidal-Carou, 2003). According to this criterion, days of difference between the control samples and the treated samples in each of the batches were established, such that the control of batch 1 was unsuitable for consumption upon arrival onshore and the treated sample was not suitable after 4 days storage on land. For batch 2, the control was unsuitable on day 4 and the treated sample on day 6. For batch 3, the control was unsuitable on day 6 yet the treated was still acceptable on day 11. Finally, both the control and treated fish from batch 4 were still suitable for consumption on day 11.

The condition of the cooked fish filets was greater for the Petfrost samples than the controls, although the differences were not always significant. In this case, half the scale is the reference value for the suitable samples, since values > 0.5 represent samples with undesirable sensory characteristics. On day 4 storage there were significant differences between the control and Petfrost samples for batches 1 and 2. In batch 3 the most prominent result corresponds to day 11 storage in which the control sample was inedible yet the Petfrost sample was still acceptable. In batch 4, all the values were < 0.5, but the differences between the control and Petfrost were significant over all the storage.

Sensory analises of raw fish and cooked fish were considered jointly. Results for raw fish were splitted in six intervals (0.05-0.2-0.35-0.5-0.65-0.8-0.950) and arithmetic means were plotted against means for sensory analises of the corresponding cooked fish. In the resulting equation ($y=0.5077\ln(x)+0.9631$; $r^2=0.9791$), a value 0.65 for raw fish corresponds to a value of 0.54 for cooked fish. This calculation has been made by excluding three cases for which no explanation between raw fish and cooked fish is found. In cooked fish, panellists noticed the largest differences in odour and texture. Odour was acid and texture had no consistency for values of 0.5. Hyldig and Green-Petersen (2004) pointed out that the sensory quality of the fish/fish product that the consumer buys depends on the quality of the raw material used for the final product sold

at retail level. The selection of the best descriptors for the spoiling fish allows sensory quality of fish to be objectively determined. This quality corresponds to a typical odour, colour, flavour and/or texture of the cooked product, which is particular for each fish species.

In summary, megrim washed with ozonised water on board and stored in the ice state by using bactericide ice flakes (both on board and on land) showed a better quality than a control fish. Also the quality of the Petfrost ice flakes was different to the control flakes, most notably, the flakes maintained their initial appearance and form for the first two weeks of refrigeration, remained loose and came apart easily, and prevented the formation for air pockets around the fish. Accordingly, the Petfrost flake ice facilitates the ice-fish contact during storage and enhances the quality of the fresh megrim. Thus, megrim treated with ozone was still suitable for consumption after 14 days on board, and megrim stored for 12, 8 and 3 days on board could be stored for five further days in the ice state once landed with an acceptable quality. In contrast, control fish was not suitable for consumption if stored for longer than three days on board. Similarly, under conditions in which both ozone-treated fish and control fish were acceptable (3 days ice-stored on board and 7 days ice-stored on land), differences were significant too, so the treatment always renders a product with a higher quality.

4. Conclusions

When megrim is washed with ozonised water on board and stored in the ice state by using bactericide ice flakes, which is called the Petfrost combined system, it can maintain a higher quality than fish treated with the traditional system (sea water for washing and making flake ice). It allows a better acceptance of megrim at the retail outlets, and increases its shelf-life.

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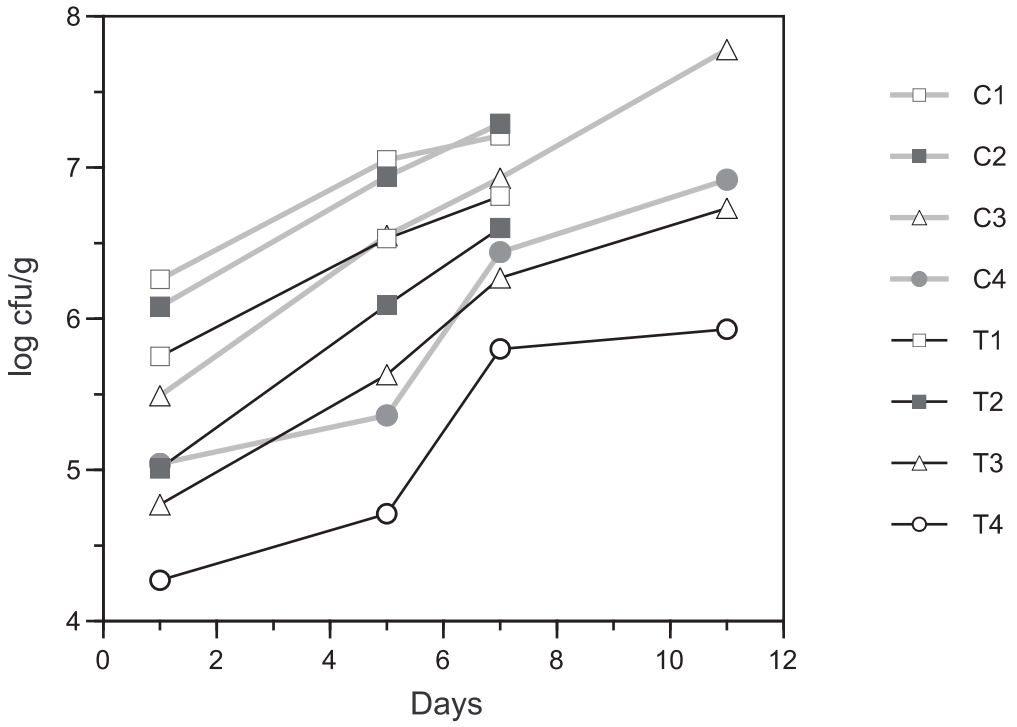
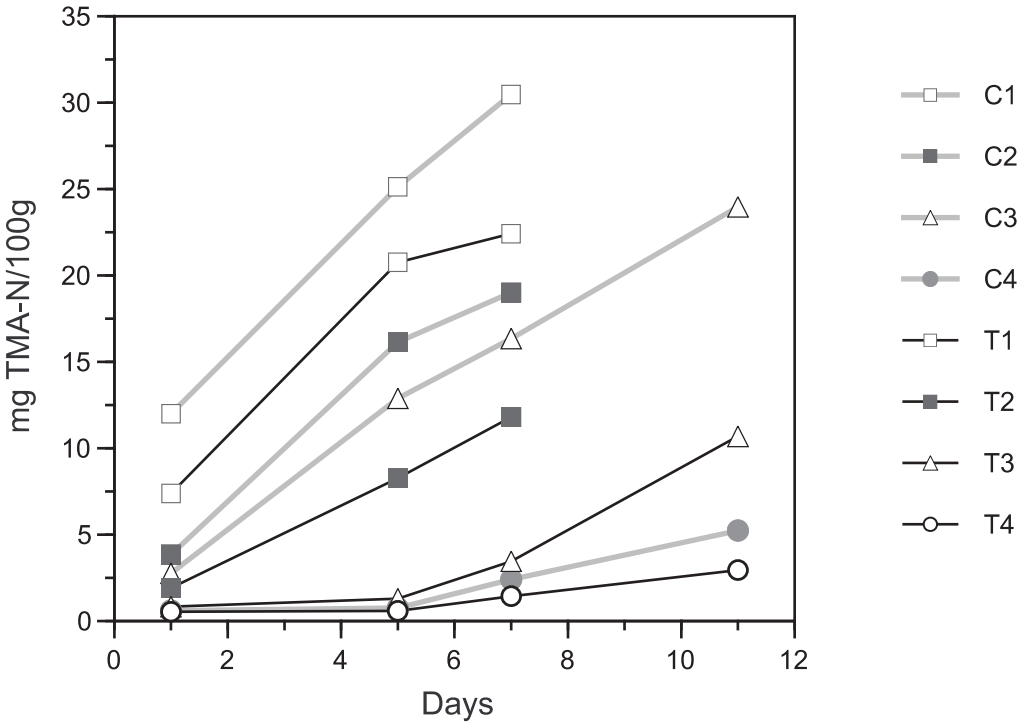
Figure(s) 1



Figure(s) 2


mg TMA-N/100g
Days
0
5
10
15
20
25
30
35
0
2
4
6
8
10
12
C1
C2
C3
C4
T1
T2
T3
T4

Figure(s) 3

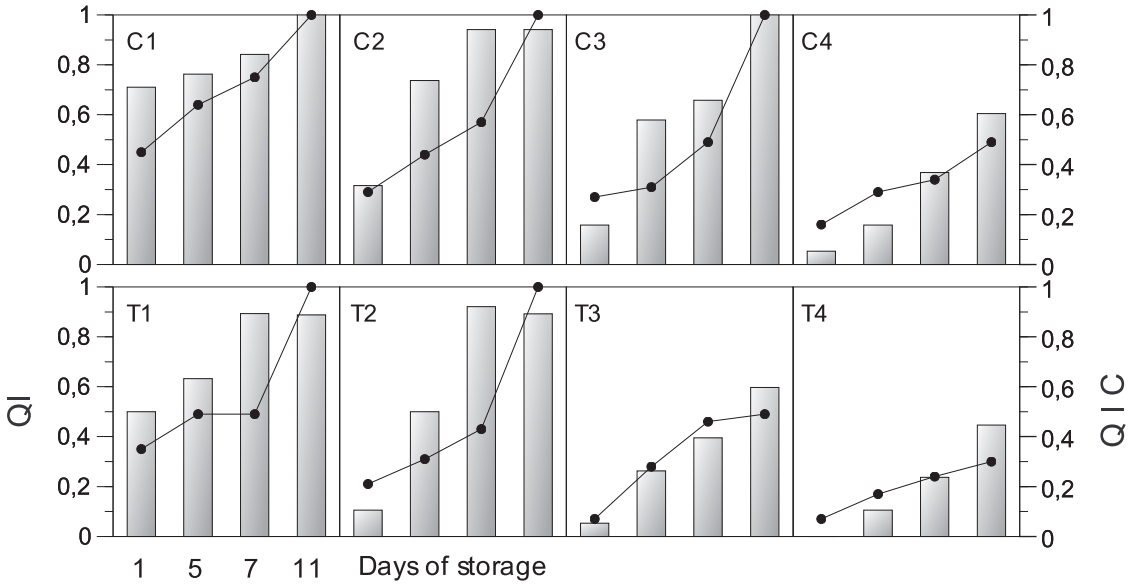


FIGURE LEGENDS

Figure 1. Evolution of total viable counts (TVC) in refrigerated megrim for Petfrost treatment (solid lines) *versus* control treatment (discontinuous lines). Samples follow the same convention; batch 1 (14 days onboard storage); batch 2 (12 days onboard storage); batch 3 (8 days onboard storage), and batch 4 (3 days onboard storage).

Figure 2. Evolution of trimethylamine nitrogen (TMA-N) in refrigerated megrim for Petfrost treatment *versus* control treatment. Sample identification follows the same convention as in Fig. 1.

Figure 3. Evolution of the sensory score for the Petfrost treatment *versus* control treatment for raw (QI in bars) and cooked (QIC in lines) megrim. C1-C5 are control batches and T1-T5 are Petfrost batches (see Fig. 1 legend).

Table(s) 1

Table 1
Sample preparation and days of storage of the batches

	Onboard		Onshore
Petfost samples:	*(Water + ice) Petfrost*	→	*Ice Petfrost*
Control samples:	*(Water + ice) Control*	→	*Ice Control*

Nº batch	Days onboard	+	Days onshore	=	Total days
1	14		1, 5, 7		15, 19, 21
2	12		1, 5, 7		13, 17, 19
3	8		1, 5, 7, 11		9, 13, 15, 19
4	3		1, 5, 7, 11		4, 8, 10, 14

Table(s) 2

Table 2

Changes in N-BVT during megrim storage in ice

Inshore days	C 1 (14)*	C2 (12)	C3 (8)	C4 (3)
1	29.64 (1.80)ka	18.30 (0.43)ka	18.06 (0.53)ka	8.30 (0.70)ka
5	38.17 (1.25)la	30.33 (1.36)la	24.12 (0.18)la	13.67 (0.91)la
7	51.26 (0.54)ma	41.69 (1.79)ma	36.00 (0.12)ma	17.72 (1.12)la
11	NA	NA	45.00 (2.24)na	24.49 (0.88)ma
Inshore days	**T 1 (14)**	**T2 (12)**	**T3 (8)**	**T4 (3)**
1	22.38 (0.84)kb	13.40 (0.58)kb	13.38 (0.34)kb	7.96 (0.59)ka
5	35.35 (2.22)la	20.85 (1.00)lb	16.63 (0.40)lb	12.46 (0.75)la
7	49.20 (1.59)ma	26.25 (0.30)mb	19.86 (1.37)lb	15.66 (0.86)la
11	NA	NA	30.65 (0.99)mb	23.62 (0.42)ma

* (days) on board. Each value represents mean (standard deviation). Values followed by letters: a-b are significantly different ($p < 0.05$) between control and "Petfrost" batches (C1:T1, C2:T2, etc); k-n between days of storage of each batch; NA is not analysed

Table(s) 3

Table 3

Change in pH values during megrim storage in ice

Inshore days	C 1 (14)*	C2 (12)	C3 (8)	C4 (3)
1	6.88 (0.11)ka	6.78 (0.06)ka	6.72 (0.08)ka	6.65 (0.06)ka
5	7.39 (0.08)la	7.22 (0.03)la	7.06 (0.04)la	6.69 (0.04)ka
7	7.87 (0.04)ma	7.62 (0.10)ma	7.58 (0.13)ma	6.75 (0.07)ka
11	NA	NA	7.87 (0.10)na	7.06 (0.13)la
Inshore days	**T 1 (14)**	**T2 (12)**	**T3 (8)**	**T4 (3)**
1	6.85 (0.08)ka	6.74 (0.04)ka	6.70 (0.03)ka	6.59 (0.04)ka
5	7.33 (0.13)la	7.00 (0.06)lb	6.79 (0.07)kb	6.61 (0.06)ka
7	7.44 (0.07)lb	7.19 (0.06)lb	6.88 (0.07)klb	6.70 (0.04)kla
11	NA	NA	7.01 (0.06)lb	6.90 (0.08)la

Each value represents mean (standard deviation). Values followed by different letters:
a-b are significantly different ($p < 0.05$) between control (C) and “Petfrost” batches (T);
k-n between days of storage of each batch; * (days) on board; NA is not analysed

Table 4
Sensory attributes quantified in raw megrim

Parameter		Characteristic	Point
DORSAL ZONE	*Aspect*	Vivid pigment	0
		Vivid pigment, but without sheen	1
		Pigment shows discoloration and lacklustre	2
		Dull, lacklustre pigmentation	3
	Mucus	Aqueous, transparent	0
		Lightly opaque	1
		Milky	2
		Yellow-grey, opaque	3
	Skin	Smooth surface	0
		Wrinkled surface	1
	Rigidity	Firm	0
		Lightly flaccid, less elastic	1
EYE	*Pupils*	Clear and black, metallic sheen	0
		Dark grey	1
		Lacklustre, grey	2
	Forma	Convex	0
		Flat	1
		Sunken, concave	2
GILLS	*Mucus*	Transparent	0
		Lightly turbid	1
		Milky	2
	Odour	Marine algae	0
		Absence of algal smell, neutral	1
		Fermented, slightly bitter	2
		Bitter, putrid	3
VENTRAL ZONE	*Colour*	White or light cream without mucus	0
		Cream or yellow with mucus	1
		Greyish colour with abundant mucus	2